Phototroph-heterotroph symbiosis for biofuels applications

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Project Goals:

The goal of this study is to establish symbiotic pairs of photoautotrophic and heterotrophic microbes in order to explore their interactions for growth and for biosynthesis of products, including metabolites and biofuels, from carbon dioxide and sunlight.

In natural ecosystems, microbes live in heterogeneous populations in which individual members contribute different functions to enhance the overall performance of the group. In our study, a cyanobacterium, *Synechococcus elongatus* or the microalgae *Chlorella* capture light and carbon dioxide via photosynthesis. Sucrose secretion has been engineered into the cyanobacteria. The sucrose is then used as a direct feedstock for either bacterial growth or growth and lipid accumulation in multiple yeast species. As previously observed [1], sucrose secretion by *S. elongatus* is strongly dependent on light, osmotic pressure, and pH, which is consistent with the sucrose/proton symport activity of heterologously expressed sucrose permease. Thus, successful pairing of *S. elongatus* and heterotrophs requires robust heterotroph growth on sucrose in an appropriate medium. In this work, we first evaluated monocultures of different phototrophic and heterotrophic species by examining different environmental parameters to guarantee a well-adapted coculture system. Of the bacteria tested, we focus on *Escherichia coli* growth. Of the four yeast species of *Cryptococcus curvatus*, *Rhodotorula glutinis*, *Yarrowia lipolytica,* and *Saccharomyces cerevisiae* investigated, the former three showed great potential to adapt the co-culture system, while *S. cerevisiae* had a high sucrose level requirement to support its growth.

Next, co-culture of phototrophs (sucrose-secreting *S. elongatus* or microalgae) and heterotrophs (bacteria or yeast) was performed, and the effect of each partner on the expansion of the symbiont was evaluated by monitoring cell numbers, final biomass, lipid production, and other culture parameters. Many of the heterotrophs can grow and efficiently utilize sucrose produced by the partner *S. elongatus* in coculture, even when sucrose levels were low in the initial growth stage. Some co-culture systems were also successfully maintained in a semi-continuous culture system. Interestingly, in some cases, the growth of the cyanobacteria *S. elongatus* cells was more robust in co-culture compared with monoculture.

These artificially established symbioses strongly support that sucrose secreted by cyanobacteria can sustain eukaryotic cell expansion. Similarly, microalgae produce nutrients that can facilitate growth of heterotrophs. Our results help shed light on the establishment of a bioenergy platform, which would combine the metabolic capacity of photoautotrophs to capture inorganic carbon and then channel the resulting organic carbon directly to heterotroph partners for producing biofuel precursors.

REFERENCES

1. Ducat, D.C., et al., *Rerouting carbon flux to enhance photosynthetic productivity.* Applied and environmental microbiology, 2012. **78**(8): p. 2660-2668.

Supported by DE-SC0012658.